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SYNTHETIC SEX PHEROMONE

for detection survey of EUROPEAN PINE SHOOT MOTH

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ABSTRACT

The sex attractant or pheromone of the European pine shoot moth is a chemical compound emitted by receptive females to "call" males for purposes of reproduction. This "come-hither" scent is now available in synthetic form and can be utilized in a very sensitive trapping device to indicate the presence or absence of the insect in a particular area.

KEYWORDS: Attractants (- pest control), European pine shoot moth, *Rhyacionia buoliana*, survey (insect).

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INTRODUCTION

The distribution of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.) has steadily increased in recent years. In addition to its native Old World distribution (Schröder 1966, deBrewer et al. 1967), the insect is now an established pest of ornamental and plantation pines in parts of North and South America (Miller 1967, Miller et al. 1970, deBrewer et al. 1967). In eastern North America, infestations are established in southern Ontario and the New England and Lake States north of 40° north latitude (Miller 1967). In western North America, infestations are primarily restricted to ornamentals in western Washington and southwestern British Columbia; however, other western locations including areas containing natural pine stands have been indicated as potentially susceptible (Daterman 1972*a*; Daterman and Carolin 1973, Carolin and Daterman 1974). In South America, *R. buoliana* infestations are established in Argentina and Uruguay (deBrewer et al. 1967). There is concern the insect will also spread to extensive plantations in neighboring Chile (Billings 1969).

In the face of these spreading infestations, it is clear that a reliable detection method is needed for monitoring the extent of the insect's distribution. Sex pheromone trapping has been shown to be more sensitive than visual techniques for detecting shoot moth infestations (Daterman and McComb 1970). The chemical structure of the *R. buoliana* pheromone has been identified (Smith et al. 1974), and the synthetic material is available for survey purposes. The purpose of this report is to outline the procedures for using the synthetic sex pheromone in traps for detection surveys.

PHEROMONE STRUCTURE AND BAIT FORMULATION

The sex pheromone of European pine shoot moth has been identified as *trans*-9-dodecenyl acetate (Smith et al. 1974). This pheromone is essentially composed of a single compound, although the synthetic preparation also contains 1.1-percent *cis*-isomer. The 98.9 percent pure synthetic material is highly attractive to the insect when released at optimal rates (Smith et al. 1974). A supply of the synthetic pheromone is now commercially available.[1/]

For trapping purposes, it is necessary to control pheromone release rates. This is because: (1) male response is highly dependent on the quantity released from a trap (Smith et al. 1974); and (2) the seasonal flight period may differ by geographic location (Daterman 1972*a*) making it desirable for a given trap to remain attractive for a period of several weeks. These requirements were met by incorporating the synthetic pheromone into a polyvinyl chloride plastic formulation. This method had previously been used to successfully control release rate of cabbage looper pheromone (Fitzgerald et al. 1973).

Steps in the pheromone-plastic formulation process are as follows:

(1) Add 49 parts (weight-basis) of the plasticizer di-2-ethyl-hexyl-phthalate plus 2 parts Advastab BC-109[2/] (antioxidant) to 49 parts polyvinyl chloride low-temperature fusing resin (Diamond PVC-74).[3/]

1/ Farchan Division, Story Chemical Corporation, Willoughby, Ohio.

2/ Cincinnati Milacron Chemicals Inc., New Brunswick, N. J.

3/ Diamond Shamrock Corp., Cleveland, Ohio.

(2) Stir the above mixture into viscous, milk-white plastisol.
(3) Add synthetic pheromone in sufficient quantity to make a 5-percent active formulation (weight basis). Example: For 10 g 5-percent pheromone-plastic formulation add 0.5 g pheromone to 9.5 g plastisol.
(4) Stir pheromone-plastisol mixture thoroughly.
(5) Evacuate air bubbles in mixture by placing in rotary evaporator under a mild vacuum for several hours (time period will vary with quantity of plastisol).
(6) Place plastisol in molds for fusing. Sections of glass-tubing work well. Pheromone-plastisol mixture can be sucked into the tube by vacuum. The bottom of the filled tube is touched down on a hotplate to seal the bottom opening of the tube.
(7) The filled tubing is placed in an oven at 145° C for 2-5 minutes or until the milky pheromone-plastisol fuses into a solid, translucent, pheromone-plastic.
(8) To remove the finished formulation from the glass molds, break the glass, run cold water into the break, and slide the glass off the plastic. The flexible pheromone-plastic rods can then be cut into desired lengths for trap lures.
(9) The pheromone formulations can be stored for an indefinite period by leaving the glass molds intact, wrapping in aluminum foil, and holding at temperatures lower than 0° F.

TRAPPING DEVICES

The commercially available, fold-open, 3M Brand Sectar I[4/] sticky trap was used exclusively in field evaluations of the synthetic pheromone (fig. 1). The advantages of these traps are their nominal cost, compactness for storage and transport, and ease of operation. The pheromone-plastic baits can simply be dropped onto a sticky surface of the opened traps, or suspended from a pin stuck through the trap (fig. 1). Different colored traps are available, but an evaluation of color preference indicated plain white traps were best for *R. buoliana* survey trapping (table 1). The Sectar 1 traps operate effectively for the shoot moth with the end "flaps" in either the open or closed position.

As an alternative to the commercial Sectar traps, a serviceable "home-made" sticky-trap can readily be constructed from pint- or quart-sized cylindrical ice cream cartons. These have been used previously in pine shoot moth sex attractant experiments (Daterman and McComb 1970). Briefly, one-half of each end (lid and bottom) of a carton is removed, the interior is coated with an adhesive such as stikem-special,[5/] the attractive bait is added, and the trap is placed in the field by suspending it by wire or string from a tree branch.

PHEROMONE DOSAGE

Figure 2 illustrates the emission of pheromone at a constant temperature (76° F) from a piece of the pheromone-plastic formulation, 3-mm-diameter by 5-mm-long. This emission rate could be varied by changing the sample shape (weight to surface-area ratio) or the concentration of the active component (Fitzgerald et al. 1973). This particular curve (fig. 2), however, is appropriate for relating pheromone dosage and male response to duration of trap effectiveness. Specific details for calculating emission

4/ 3M Brand Sectar I adhesive traps now available from Zoecon Corp., 975 California Ave., Palo Alto, Calif.

5/ Michel and Pelton Co., Emeryville, Calif.

Figure 1.--Adhesive trap showing captured *R. buoliana* males and pheromone-plastic bait suspended on pin (arrow).

Table 1.--*Effect of trap color on response of male European pine shoot moth to Sectar I traps uniformly baited with synthetic pheromone*

Item	Trap color		
	White	Fluorescent yellow	Dark green
Mean number males per trap[1/][2/]	39.13 (± 9.2)	3.25 (± .8)	17.38 (± 3.7)

1/ Each treatment replicated 8 times.

2/ Standard error of mean in parentheses.

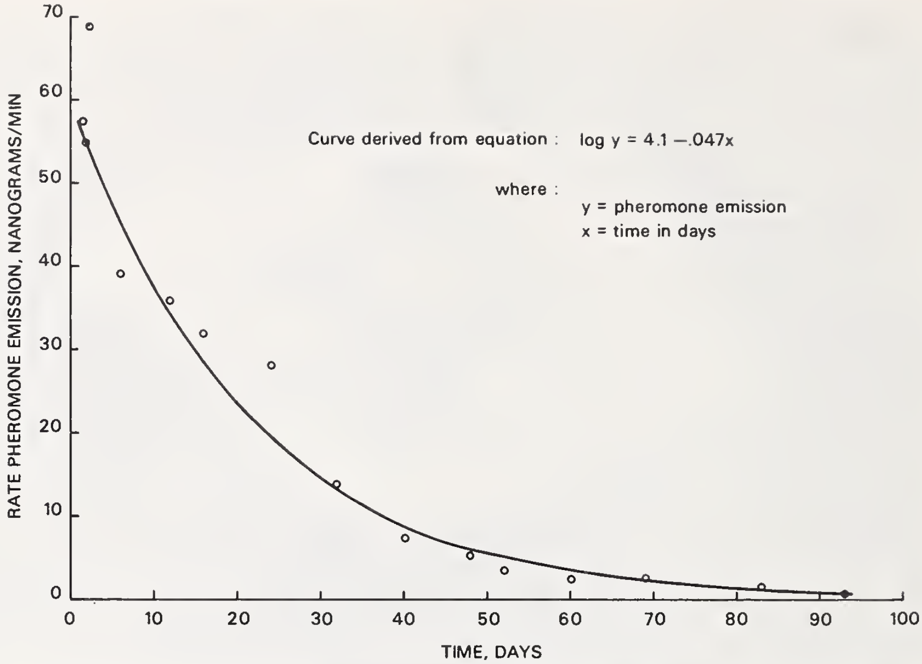

Figure 2.--Rate of pheromone emission from 5-mm-long by 3-mm-diameter pheromone-plastic section (5-percent active). Plotted points determined by weight-loss analysis at constant temperature of 76° ± 1° F.

rates of pheromone from plastic formulations have been given by Fitzgerald et al. (1973) and Smith et al. (1974).

Figure 3 summarizes dosage response to different pheromone release rates. The synthetic baits are clearly more attractive than the live females. It is significant to note that even after an exposure period of 3 months, at a constant 76° F temperature, the synthetic bait would still be considerably more attractive than live females (figs. 2 and 3).

Figure 3 data were obtained by baiting traps with different sized pieces of the 5-percent pheromone-plastic formulation and exposing the traps in the field for four consecutive days and nights. The three most effective dosages, averaging 5, 33, and 56 nanograms per minute, were not significantly different from one another at $p = 0.05$ (Smith et al. 1974). Thus, by relating the information in figures 2 and 3, we see that a 5-percent active, 3-mm-diameter by 5-mm-long section of pheromone-plastic will maintain optimal release rates of 5-56 nanograms pheromone per minute for a period of almost 50 days at a constant temperature of 76° F. This residual capability of the baits should simplify operational survey work since some of the traps can be placed in the field in advance of the actual flight season and bait recharging will not be necessary.

The curve in figure 2 is derived from data obtained at a constant temperature regime; hence local conditions at field-trapping locations could greatly influence pheromone loss rates. To evaluate this aspect, samples of the pheromone-plastic formulation were exposed (in traps) outside the Corvallis laboratory for 8-day periods during "cool" and "hot" summer weather. The "cool" period was characterized by intermittently cloudy and some clear weather with daily maximum

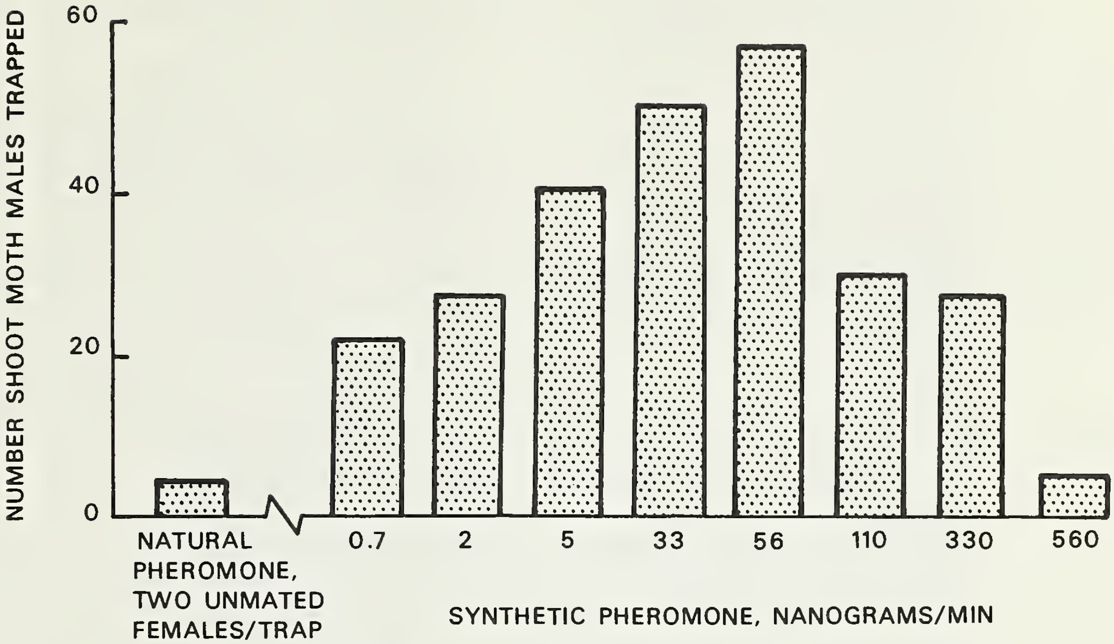

Figure 3.--Field attraction of *R. buoliana* males to natural and synthetic pheromone baits. (Six replicates per treatment; response to three best treatments not significantly different at p = 0.05).

temperatures of 68°-74° F. The "hot" period was characterized by mostly clear skies and daily maxima of 80°-85° F. During the first 8 days of exposure, the laboratory sample (constant 76° F) had a net loss of 29 percent of its pheromone charge, whereas the "outdoor" samples lost 18 percent during "cool" weather conditions and 37 percent during 8 days of "hot" weather. Based on the above, the 3-mm-diameter by 5-mm-long pheromone-plastic section would emit attractant at optimum levels for at least a 5- to 6-week period even during periods of continuing hot weather.

The emission rate of pheromone from the controlled-release formulation is very closely approximated by the equation:

$$\log y = 4.1 - .047\, x$$

where x = time in days
y = pheromone emission (nanograms/min)

The curve in figure 2 expresses this relationship graphically. Such curves will change with temperature, but have considerable value for predicting the active life of pheromone baits for trapping programs.

TRAP PLACEMENT

Once trapping devices and pheromone baits have been assembled, questions arise concerning the number of traps per unit area that are necessary for survey. In natural forests or plantations, the number of traps is determined by the effective pheromone response distance by male moths. Response distance estimates are difficult because of the number of influencing factors and the extreme variability of the biological parameters involved. Nevertheless, realistic estimates can be made; and when considered from this point of view, the values listed below should provide sound guidelines for trap placement.

Distance response estimates for *R. buoliana* males are based on the Bossert and Wilson (1963) formula to calculate the theoretical mean maximum distance of pheromone communication in moving air. This formula with supplemental variations was also used by Sower et al. (1971, 1973) to calculate response distance of the cabbage looper, *Trichoplusia ni*. The formula is:

$$X = \left(\frac{8Q}{vK}\right)^{4/7}$$

where: X = response distance (cm)
Q = pheromone emission rate (μg/sec)
K = pheromone response threshold ($\mu g/cm^3$ of air)
v = wind velocity (cm/sec)

The numerical constants in the formula are concerned with dispersion of a gas as influenced by wind velocity. For discussion of this topic, see Sutton (1953) and Bossert and Wilson (1963).

The estimate of K was based on weight-loss of synthetic pheromone-plastic formulation (0.5 percent active) and accompanying bioassays of small samples of the formulation. This method yielded a K estimate of 4.5×10^{-11} $\mu g/cm^3$. The method of bioassay was the same used to quantify male responses to female equivalency fractions of the natural pheromone in a prior study (Daterman 1972*b*). This K value approximates an average pheromone response threshold, since 40-50 percent of the test males responded to this level of pheromone emission.

Under favorable conditions (low, steady wind velocity of 0.1 mi/h), average male communication distance to an optimal dosage of the synthetic pheromone could reach 198 meters or 0.12 mile. A small percentage of males have a lower pheromone response threshold and thus could respond over even longer distances.

Wind velocity is extremely important. At higher wind velocities, potential response distance decreases because of (1) a more dilute initial concentration of pheromone near the emission source and (2) a more rapid gas dispersion due to more turbulence (Sutton 1953). Figure 4 illustrates the pheromone response distance for *R. buoliana* at various wind velocities for two levels of pheromone dosage. The highest dosage level, which corresponds to a fresh bait, has the capacity of drawing males 25-200 m (80-650 ft) with steady wind conditions ranging from 0.1 to 4.5 mi/h. The lower dosage level, which corresponds to a pheromone bait that has been in the field for 6-8 weeks, could attract males a distance of 6-50 m (20-160 ft) under the same wind conditions. Upwind flight of *R. buoliana* ceases at wind speeds of 6 mi/h or higher (Green and Pointing 1962) which would cause distance response to the pheromone to break down at that point.

With these dimensions in mind, it is possible to construct a grid pattern of pheromone traps that is applicable for survey of large land areas. One trap per 4 acres should be highly efficient for detection of shoot moth infestations in such areas as forest or Christmas tree plantations, forest regeneration lands, or large nursery plantings. Figure 5 illustrates the effective attraction distance of synthetic baits that have been in the field for various periods of time. Since a given male shoot moth would remain available for capture over a period of 10 days to 2 weeks and would itself move around within the survey area, the degree of coverage indicated by figure 5 should be very efficient for detection purposes. The 0.5-mi/h wind velocity used as a basis for calculation of distance response values in figure 5 may appear somewhat

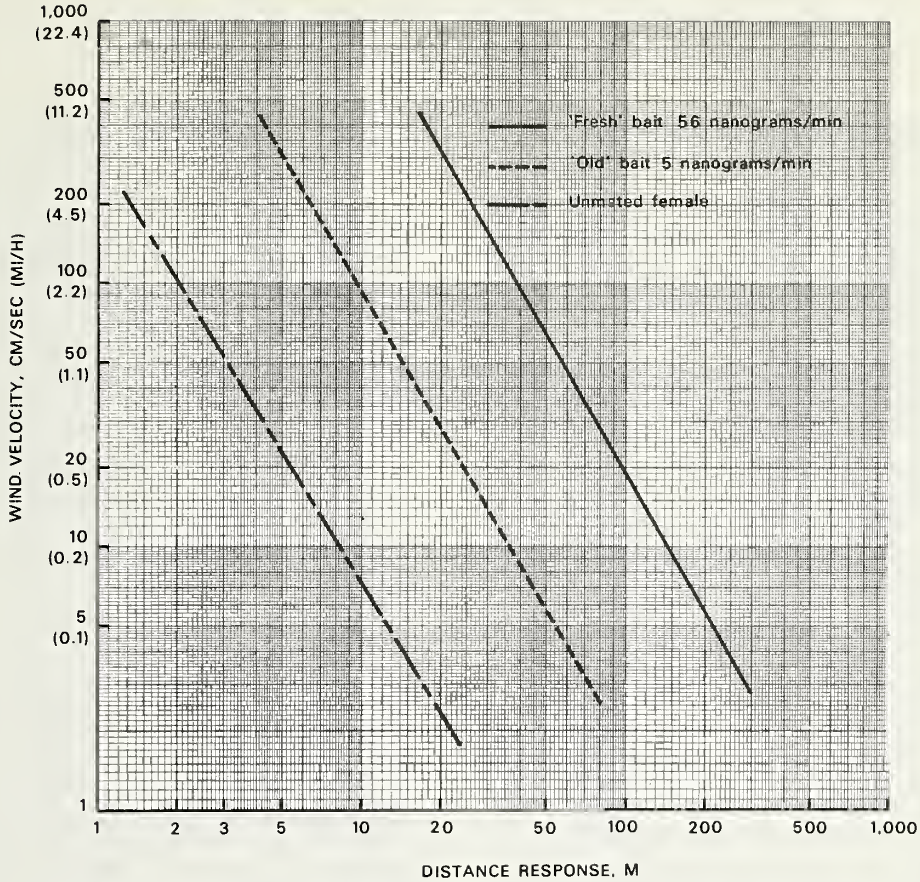

Figure 4.--Effects of different wind velocities and dosage on potential pheromone response distance by *R. buoliana* males (temperature 76° ± 1° F).

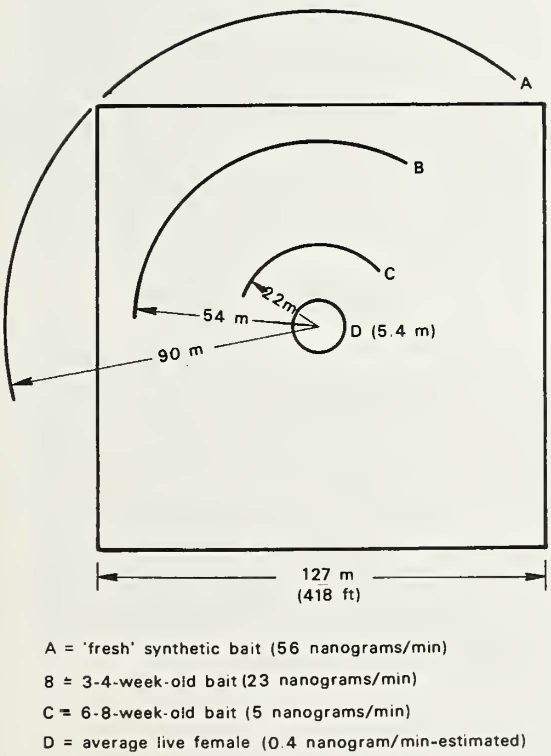

Figure 5.--Effective pheromone communication distance (radii) of various *R. buoliana* pheromone baits at a steady wind speed of 20 cm/sec (0.5 mi/h), in relation to a 1.6-hectare (4-acre) land area (constant temperature 76° ± 1° F).

low; however, this type of gentle wind action is not uncommon during summer evenings. If stronger evening winds predominate in a particular area, the number of traps per unit land area must increase accordingly (fig. 4).

Where it is necessary to survey residential areas, parks, golf courses, etc., the pheromone response distance indicated in figures 4 and 5 do not necessarily apply. The presence of buildings, movement of humans, automobile traffic, and artificial lighting will no doubt have some effects on flight behavior and pheromone response of males. The moths might not, for example, cross open or lighted areas in response to the pheromone signal. The best policy in this situation is to place traps in as many individual pine plantings as resources will permit for a given residential area.

Previous experience (Daterman and McComb 1970) has proven that individual traps are most effective if placed on host trees. Flying males orient around the periphery of the top half of the crown of host trees. This is not surprising since receptive females most frequently locate themselves on pine needles in the outer edge of the crown. Therefore, traps should be placed toward the top or upper one-third of the foliage crown of the pine. Also, traps should be situated on the outer periphery of the pine foliage, as opposed to being hidden beneath the branches. For taller host trees, a trap height of 5-7 feet above ground is adequate. When host plants are small and fragile, it might be necessary to place the traps on stakes or some other support. Even in these situations, however, the traps should be placed directly adjacent to the host plant and preferably touching the foliage.

SEASONAL TIMING

The residual capacity of the controlled-release pheromone-plastic baits greatly simplifies the problem of placing traps in the field coincident with the onset of seasonal flight. Even if traps are put out a month or more prior to adult emergence, the baits will still be highly attractive (figs. 2 and 3). In spite of this obvious advantage, however, the fresher the trap, the more efficient the bait (figs. 4 and 5). Therefore, trap placement should coincide as nearly as possible with the beginning of the seasonal flight period.

For infestations in western Washington and northwestern Oregon, adult flight usually begins during early June; and pheromone survey traps should be in place for these areas by June 5. For southwestern Oregon and lower elevations east of the Cascade Mountains in Oregon and Washington, *R. buoliana* flight may be in progress by mid-May. For these areas, traps should be in place by May 10.

For higher elevations (> 4,000 ft) east of the Cascade summit, spring development of *R. buoliana* is slower (Daterman 1972 *a*) and flight will not occur until late June or July. For such locations, pheromone traps should be in place by June 20.

CAPTURED MOTHS

Any moths captured in pheromone traps should be saved for examination by personnel knowledgeable in species identification. Although lepidopteran pheromone responses are rather specific, some cross-attraction between species can occur. Since the finding of new infestations can have far-reaching economic implications, any captured specimens should be saved for positive identification.

If left in the sticky traps, captured moths eventually (approximately 30 days) become saturated with the adhesive compound which causes a loss of their distinctive color patterns. When this occurs the specimens can be recovered by soaking in hexane. Before soaking, trapped specimens should be placed in a "relaxing" container (humid environment) for a period of 36-48 hours. The "relaxing" procedure is necessary to prevent damage to the captured moths which become dry and brittle after long periods in the traps.

OTHER POTENTIAL APPLICATIONS

Because of the general effectiveness of the synthetic pheromone formulation, other than survey uses should be considered. In areas where the shoot moth is an established pest, for example, pheromone traps might be used to time the application of control agents. If administered by regulatory agencies, particular areas or locations could be certified as being free of *R. buoliana* infestation following an intensive area-wide survey-trapping program. Finally, population control by pheromone applications may be possible by the "trapping-out" or "male-annihilation" techniques (Knipling and McGuire 1966), or by mating disruption (Shorey et al. 1967). The latter application is being investigated.

SUMMARY AND CONCLUSIONS

(1) The sex pheromone of European pine shoot moth has been chemically identified as *trans*-9-dodecenyl acetate. The active synthetic material contains 1.1 percent *cis*-isomer.

(2) The synthetic pheromone has been formulated in a solid-plastic matrix to control emission in the field. In comparison to live females these synthetic baits are substantially more attractive to male moths.

(3) A 5-mm-long by 3-mm-diameter section of the pheromone-plastic formulation (5-percent active) represents an optimal dosage for attraction to adhesive traps. The bait section can be suspended from a pin stuck through the top of the trap, or simply dropped onto the sticky surface of the floor or sides of the open trap.

(4) The effective distance of male response to the traps depends on many variables. With optimal wind conditions, the synthetic baits are capable of attracting males a distance of 198 meters (650 ft).

(5) Over large uniform plantings such as forest lands, nurseries, or pine plantations, one trap should be allocated per 4 acres. For ornamental plantings in residential areas, traffic, artificial lighting, and other disturbances can disrupt normal male responses to the pheromone signal. The best policy in such areas is to place traps in as many individual pine plantings as resources will permit.

(6) Any captured moths should be saved for positive identification.

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